1.6.2015

MONDAY, 6/1

Only the transformations for AH005 and AH013 were successful.
We did o/n culture of those.

Created 500 ml of SOB with the following substances:

Per 500 ml:

- 2,5 g yeast extract
- 10 g tryptone
- 0.292 g NaCl
- 0.093 g KCl
- 1,2 g MgSO4

Added all the integredients in same bottle before autoclave.

Re-ligating the restricted plasmids of AH011, AH013 and AH014.
8µl Restricted DNA
1µl 10x T4 Ligase Buffer
0,5µl T4 Ligase
0,5µl H2O
-> 30 mins in rt
-> 20min inactivation in +80°C
->+4°C

-> used 2µl of this ligation mix to transform TOP10 competent cells
-> In addition, made new transformations of the old ligations (from friday) and used 8µl of ligation mix for each transformation.
-> Followed the transformation protocol
-> plated 100µl of each transformant on km plates
-> +37°C o/n

o/n culture of TOP10 cells to prepare more competent cells

- Prepared more 0,1M CaCl2 solution
    - 5ml 1.0M CaCla2 + 45ml sterile H2O

Made an o/n culture of AH009 (a backbone with chloramphenicol resistance)

- 2 ml LB, 34 mg/ml choramphenicol

2.6.2015

TUESDAY, 6/2

Refreshed AH009: added 100ul o/n culture into a fresh 2ml tube. Incubation started at 10:10. Plates were made at 14:10 (5,10,20,50 and 100 ul) after 4h and 16:40 after 6h: plated the amounts of refreshed culture listed above on chloramphenicol plates after 4h and 6h incubation.

Started making TOP10 chem comps and followed the protocol. Incubation (37C) with shaking started at 10:40. At 12:10 OD was 0,236 (12:10). Reincubating started at 12:29 and ended at 12:39 (OD 0,352). 12:47. Followed the protocol, but the first ice cool down period was 20 min and second 38 min. In step 13, 110µl aliquots were made
-> stored in -80°C freezer

Minipreps for AH005 and AH013
-followed ThermoSci GeneJET plasmid Miniprep Kit (Kit protocol can always be found with the kit)
-2 ml o/n cultures -> 2ml tubes
-No changes to protocol

The following results were measured with NanoDrop:

Table1

Sample	Concentration (ng/µl)	A268/280
AH005	52,2	1,90
AH013	65,9	1,88

Did o/n cultures in 2 ml LB following Table 2:

Table2

Colony	Antibiotic	Volume (µl)
AH001	Chloramphenicol	1,5
AH002	Chloramphenicol	1,5
AH003	Chloramphenicol	1,5
AH004	Chloramphenicol	1,5
AH005	Ampicillin	1,0
AH006	Chloramphenicol	1,5
AH008	Kanamycin	0,8

We restricted AH001, AH002, AH004, AH005, AH006 and AH008 following the protocol and guidelines (post-its) most of the time, but because of mess up in each restriction tube were:

0,5 µl / 1 enzyme
2,5 µl CutSmart Buffer
x µl H2O
x µl DNA (maybe around 3500 ng)

Did ligations according to protocol, but with different amounts of restriction mixes:
20 µl complete volume:

1 µl ligase
2 µl ligation buffer
5,66 µl of each restriction mix, totaling to 16,98 µl
0 µl H_2O

3.6.2015

WEDNESDAY, 6/3

Present: Anna, Petra, Tamannae, Tuukka

Made a 1% agarose gel to run yesterday's ligations. The agar we've used before had run out so we had to use another agar which was NuSieve LGT agarose. The produced gel was too fragile, so we decided not to use that one again.
The second gel batch (1,5 %, a recommendation) was made of Fermentas TopVision LE QC agarose.

2x restriction-digestion of the ligations. Restricted the ligations with EcoRI following the protocol.
-Because uncertainty of the last incubation (8OºC for 20 min), I incubated 25 µl of the restriction mix extra 10 minutes and another 25 µl extra 20 minutes. Before extra incubation, the tubes were in the ice about 25 minutes.
-> we had six samples after this:
168-10min
168-20min
248-10min
248-20min
258-10min
258-20min
*1,2,4,5,6,8 indicate the numbers we have given to our constructs: 1=A001, 2=AH002 etc.)

When pipetting the samples to the gel the wells were leaking so we were able to run only 168-10min and 168-20min samples.

The gel was so fragile that it broke down after running. The part where our desired DNA didn't break but we weren't able to cut any DNA out of it. To see the bands better the leftovers of the gel were covered with the buffer and a drop of ETBR for 20 min (15-30min is what was recommended).
No use -> discarded the gel :(

A new gel was made for the rest of the samples. We decided to run the leftovers of 168-10min and 168-20min samples to see if we could isolate some DNA out of them.

The gel
Tested transformation efficiency of new competent TOP10 cells with transformation efficiency kit 50 pg and 20 pg DNA samples and #37 control. Included water control. Followed the protocol. Plated 50 ul of transformed TOP10 to every plate, 1 plate per sample.

MiniPreps for AH001, AH002, AH003, AH004, AH005, AH006, AH008. AH008 did not grow in the o/n culture.

Table1

Sample	Concentration (ng/µl)	A260/280
AH001	51,4	2,03
AH002	46,9	2,01
AH003	58,3	2,09
AH004	43,2	2,04
AH005	44,8	2,02
AH006	55,0	1,98
AH008	0,7	1,69

Started making transformations for AH020 (VioE) and AH021 (BglX) (1ul DNA / 50 ul competent TOP10 cells) following the protocol. However, the first incubation on ice lasted 35 minutes.
Plated 20ul and 100 ul of both samples on chloramphenicol plates. No amp control was made.

Plates left to incubator at 13.26

Started making transformations for AH008 (a backbone with kanamycin resistance) (1ul DNA / 50 ul competent TOP10 cells). First incubation on ice started at 14:25. The final incubation started at 15.38. Plated the transformants on kanamycin plates.

Isolated the correct DNA pieces from the 2nd gel. Purified DNA with Thermo Scientific gel extraction kit. Followed the kit protocol, added Binding buffer according to the table 2:

Table2

248 10	**248 20**	**258 10**	**258 20**	**168 10+20**
97,6	56,6	92,8	111,6	11,6

Ligated 8 µL the purified DNA. Followed the ligation protocol. Stored the ligations and the purified DNA in the fridge.

4.6.2015

THURSDAY, 6/4

Refreshed AH008 on a fresh kanamycin plate.

Transformed yesterday's ligations: 168 (AH011), 248-10, 248-20 (AH014), 258-10 and 258-20 (AH015). Followed the protocol. Transformants were plated to 25 ug/ml kanamycin plates made by Petra during the incubation (no kan plates in the fridge). The plates were made following the protocol: 100 ml agar, 50 ug kanamycin stock (50 mg/ml).

Transformed AH020 (Plate 2, 1E) & AH021 (Plate 4, 3G) into TOP10 according to transformation protocol.

5.6.2015

TUESDAY, 8/4

Checked yesterday's transformations:

- Not successful: 168 (AH011), 248-10, 248-20 (AH014), 258-10 and 258-20 (AH015).
- Successful: AH020 (VioE) & AH021 (BglX)